OncoScan™ CNV Plus Assay—7 Samples

Catalog Numbers 902294 and 902292

Pub. No. MAN0029389 Rev. A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *OncoScan™ CNV Plus Assay User Guide* (Pub. No. MAN0027850). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! This quick reference is intended for users who are familiar with the OncoScan™ CNV Plus Assay.

This quick reference differs from the user guide. It does not contain as much detail and does not contain the optional stoppint points. Users must be familiar with the assay and the user guide before using this quick reference.

General procedures

Laboratory

- The Post-PCR Room has airborne contamination with the PCR amplified MIP-annealed template. After entering the Post-PCR Room, do not re-enter the Pre-PCR Room.
- Do not open the seal of the **1st PCR Plate** in the Pre-PCR Room.
- Change gloves frequently throughout the assay and when instructed.
- Store reagents in the appropriate Pre-PCR or Post-PCR Room as detailed on the packaging. Only store the reagents used for Anneal, Gap Fill, and 1st PCR in the Pre-PCR Room.
- Do not mix and match reagents from other reagent kits.
- Properly chill equipment such as cooling blocks and reagent coolers before use.
- Unless otherwise indicated, keep all reagents on ice, or on a chilled cooling block on ice.
- Ensure that the enzymes are kept at –25°C to –15°C until needed. When removed from the freezer, immediately place in a benchtop reagent cooler that has been chilled to –25°C to –15°C.
- Reagents are always added to a chilled PCR plate on the chilled cooling block.
- Whenever a chilling step is called for, chill the plate on the chilled cooling block for 1 minute, then centrifuge before adding reagents.
- The thermal cycler protocols are multi-stepped and include pausing the protocol, performing a step, then resuming the protocol. Ensure protocols are paused and resumed properly.

Vortexing

- Reagent vials: Vortex 3 times, 1 second each time.
- Enzyme vials: Vortex for 1 second, 1 time.
- Master mix tubes: Vortex 3 times, 1 second each time. Do this before and after adding an enzyme.
- Vortex plates: Vortex 3 seconds in all corners and in the center.
- Always ensure that the plates are tightly sealed. A tight seal prevents sample loss and cross-well contamination, particularly when plates are being vortexed.

Centrifuging

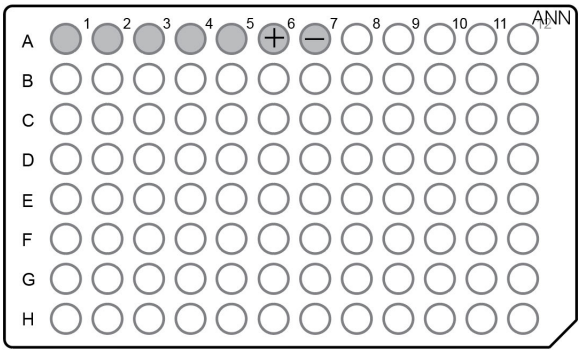
- Reagent vials: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Enzyme vials: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Master mix tubes: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Strip tubes: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Plates: Centrifuge for the specified time at 2,400 rpm using a plate centrifuge.

For Research Use Only. Not for use in diagnostic procedures.

Prepare the Sample Plate

Perform the following steps in the Pre-PCR Room.

1. Label a 96-well plate “Anneal” and place it on a cooling block. This plate is called the **Anneal Plate**.
2. Thaw the Normalized Test gDNA samples (12 ng/µL), Positive Control (12 ng/µL), and Negative Control.
3. Once thawed, vortex, centrifuge, and place them on ice.
4. Add 6.6 µL of Normalized Test gDNA sample to wells A1—A5 of the **Anneal Plate**.
5. Add 6.6 µL of the Positive Control to well A6 of the **Anneal Plate**.
6. Add 6.6 µL of the Negative Control to well A7 of the **Anneal Plate**.
7. Seal the **Anneal Plate**, centrifuge for 30 seconds, then place it on a cooling block.



Stage 1: Anneal

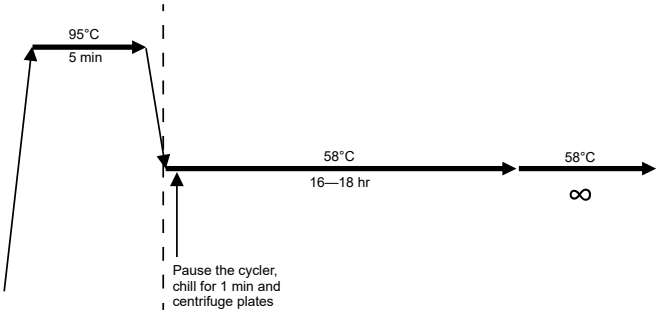
Perform this stage in the Pre-PCR Room.

1. Power on the thermal cycler.
2. Fill the ice bucket with ice. Place 2 cooling blocks on ice.
3. Thaw the Buffer A, Copy Number Probe Mix 1.0, and Somatic Mutation Probe Mix 1.0.
4. Once thawed, vortex, centrifuge, then place them on ice.
5. Label a 1.5-mL tube “ANN” and place it on ice.
6. Label a 7-well strip tube “ANN” and place on a cooling block.
7. Prepare the Anneal Master Mix in the “ANN” 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 1 Anneal Master Mix.

Reagent and cap color	1 reaction	7 reactions (~60% overage)
◯ Buffer A	1.53 µL	17.1 µL
● Copy Number Probe Mix 1.0	1.37 µL	15.3 µL
◯ Somatic Mutation Probe Mix 1.0	0.5 µL	5.6 µL
Total volume	**3.40 µL**	**38.0 µL**
Strip tube wells		**7**
Volume per strip tube well		5.0 µL

8. Vortex and centrifuge the Anneal Master Mix, then place it on ice.
9. Aliquot 5.0 µL of the Anneal Master Mix into wells 1—7 of the “ANN” strip tube, then place it on a cooling block.
10. Remove the seal from the **Anneal Plate**.
11. Transfer 3.4 µL of Anneal Master Mix from the “ANN” strip tube to the **Anneal Plate**. Pipet up and down to 3 times to rinse the tips.
12. Seal the **Anneal Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.
13. Load the **Anneal Plate** into the thermal cycler and start the OncoScan Anneal protocol.



14. After 6 minutes, pause the protocol at 58°C.

    Note: Ensure that the thermal cycler protocol is paused.
15. Remove the **Anneal Plate** from the thermal cycler and place it on a cooling block for 1 minute. Centrifuge for 30 seconds, then place it on a cooling block
16. Load the **Anneal Plate** into the thermal cycler then resume the OncoScan Anneal protocol.

    Note: Ensure that the thermal cycler protocol has resumed and is running.
17. Allow the OncoScan Anneal protocol to run overnight, 16—18 hours.

Stage 2: Gap Fill through 1st PCR

Perform this Stage in the Pre-PCR Room.

1. Fill the ice bucket with ice. Place 4 cooling blocks on ice.
2. Thaw the Nuclease-Free Water, dNTP Mix (A/T), dNTP Mix (G/C), Buffer A, Cleavage Buffer, and PCR Mix.
3. Once thawed, vortex, centrifuge, then place them on ice.

Stage 2A: Prepare the AT and GC Master Mixes

1. Label a 1.5-mL tube "AT" with a blue marker. Label a 1.5-mL tube "GC" with a red marker. Place them on ice.
2. Label a 7-well strip tube "AT" with a blue marker. Label a 7-well strip tube "GC" with a red marker. Place them on separate cooling blocks.
3. Prepare the AT Master Mix in the "AT" 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 2 AT Master Mix.

Reagent and cap color	1 reaction	7 reactions
◯ Nuclease-Free Water	3.93 µL	118.0 µL
dNTP Mix (A/T)	0.07 µL	2.2 µL
Total volume	**4.0 µL**	**120.2 µL**
Strip tube wells		**7**
Volume per strip tube well		9.6 µL

4. Vortex and centrifuge the AT Master Mix, then place it on ice.
5. Aliquot 9.6 µL of the AT Master Mix into wells 1—7 of the "AT" strip tube. Seal the strip tube, then place it on a cooling block.

IMPORTANT! Discard the gloves used to prepare the AT Master Mix. Don fresh gloves to prepare the GC Master Mix.

6. Prepare the GC Master Mix in the "GC" 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 3 GC Master Mix.

Reagent and cap color	1 reaction	7 reactions
◯ Nuclease-Free Water	3.93 µL	118.0 µL
dNTP Mix (G/C)	0.07 µL	2.2 µL
Total volume	**4.0 µL**	**120.2 µL**
Strip tube wells		**7**
Volume per strip tube well		9.6 µL

7. Vortex and centrifuge the GC Master Mix, then place it on ice.
8. Aliquot 9.6 µL of the GC Master Mix into wells 1—7 of the "GC" strip tube. Seal the strip tube, then place it on a cooling block.

IMPORTANT! Discard the gloves used to prepare the GC Master Mix. Don fresh gloves to prepare the Gap Fill Master Mix.

Stage 2B: Prepare the Gap Fill Master Mix

1. Label a 1.5-mL tube "G" and place it on ice.
2. Label a 7-well strip tube "G" and place it on a cooling block.
3. Remove the SAP, Recombinant (1 U/µL) and the Gap Fill Enzyme Mix from the freezer, then place them in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place them in the cooler.
4. Prepare the Gap Fill Master Mix in the "G" 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 4 Gap Fill Master Mix.

Reagent and cap color	1 reaction	7 reactions (~20% overage)
◯ Nuclease-Free Water	10.58 µL	89.0 µL
◯ Buffer A	1.18 µL	9.9 µL
SAP, Recombinant (1 U/µL)	0.84 µL	7.1 µL
Gap Fill Enzyme Mix	1.40 µL	11.8 µL
Total Volume	**14.0 µL**	**117.8 µL**
Strip tube wells		**7**
Volume per strip tube well		16.0 µL

5. Vortex and centrifuge the Gap Fill Master Mix, then place it on ice.
6. Aliquot 16.0 µL of the Gap Fill Master Mix into wells 1—7 of the "G" strip tube, then place it on a cooling block.

Stage 2C: Add the Gap Fill Master Mix

1. After 16—18 hours, remove the **Anneal Plate** from thermal cycler, and place it on a cooling block for 1 minute. Centrifuge for 30 seconds, then place it on a cooling block.

2. Stop the OncoScan Anneal protocol. Start the OncoScan Gap Fill protocol. When the temperature reaches 58°C, pause the protocol.

   Note: Ensure that the thermal cycler protocol is paused.

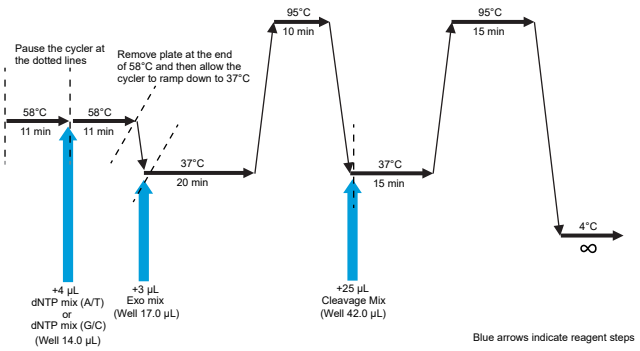


Figure 1 OncoScan Gap Fill thermal cycler protocol.

3. Remove the seal from the **Anneal Plate**.

4. Transfer 14.0 µL of Gap Fill Master Mix from the "G" strip tube to the **Anneal Plate**. Pipet up and down to 3 times to rinse the tips.

5. Seal the **Anneal Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.

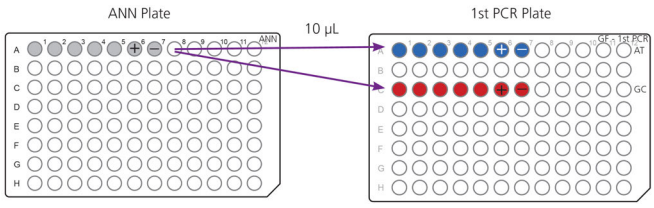
Stage 2D: Perform the Channel Split

1. Label a 96-well plate "1st PCR" and place it on a cooling block. This plate is called the **1st PCR Plate**.

2. Label wells A1—A7 with a blue marker and wells C1—C7 with a red marker.

3. Remove the seal from the **Anneal Plate**.

4. Transfer 10.0 µL of each sample from wells A1—A7 of the **Anneal Plate** to wells A1—A7 and C1—C7 of the **1st PCR Plate**.



5. Seal the **1st PCR Plate**, centrifuge for 30 seconds, then place it on a cooling block.

6. Load the **1st PCR Plate** into the thermal cycler then resume the OncoScan Gap Fill protocol.

   Note: Ensure that the thermal cycler protocol has resumed and is running.

7. Start a timer for 10 minutes.

8. After 11 minutes, pause the protocol at 58°C.

   Note: Ensure that the thermal cycler protocol is paused.

9. Remove the **1st PCR Plate** from the thermal cycler and place it on a cooling block for 1 minute. Centrifuge for 30 seconds, then place it on a cooling block.

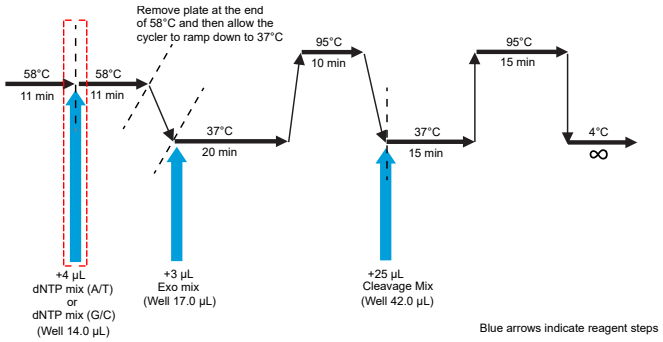


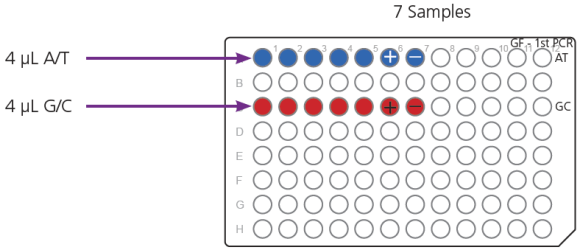
Figure 2 OncoScan Gap Fill thermal cycler protocol.

Stage 2E: Add the AT and GC Master Mix

1. Remove the seal from the **1st PCR Plate**.

2. Transfer 4.0 µL of AT Master Mix from the "AT" strip tube to wells A1—A7 of the **1st PCR Plate**. Pipet up and down to 3 times to rinse the tips.

3. Transfer 4.0 µL of GC Master Mix from the "GC" strip tube to wells C1—C7 of the **1st PCR Plate**. Pipet up and down to 3 times to rinse the tips.



4. Seal the **1st PCR Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.

5. Load the **1st PCR Plate** into the thermal cycler then resume the OncoScan Gap Fill protocol.

   Note: Ensure that the thermal cycler protocol has resumed and is running.

6. Start a timer for 10 minutes.

IMPORTANT! When the timer ends, return to the thermal cycler to pause the protocol. It is critical to pause and remove the plate from the thermal cycler at the end of the 58°C incubation. ***Do not*** allow the plate to ramp down to 37°C.

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7. Prepare the Exo Mix.

Stage 2F: Prepare and add the Exo Mix

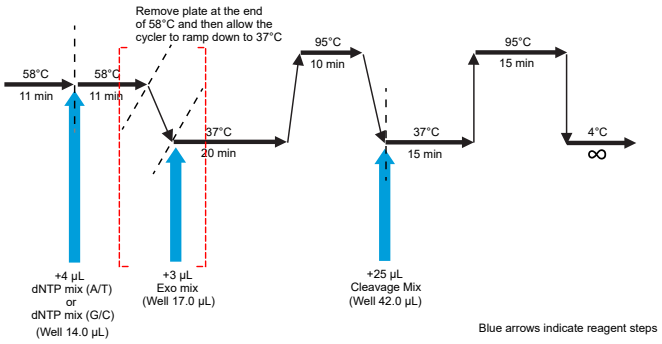
1. Label a 7-well strip tube “EXO” and place it on a cooling block.

2. Remove the Exo Mix from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.

3. Aliquot 7.5 µL of Exo Mix into wells 1—7 of the “EXO” strip tube, then place it on a cooling block.

4. After 11 minutes, pause the protocol at the end of the 58°C incubation.

IMPORTANT! It is critical to remove the plate before the thermal cycler protocol begins ramping down to 37°C.



5. Remove the **1st PCR Plate** from the thermal cycler and place it on a cooling block for 1 minute.

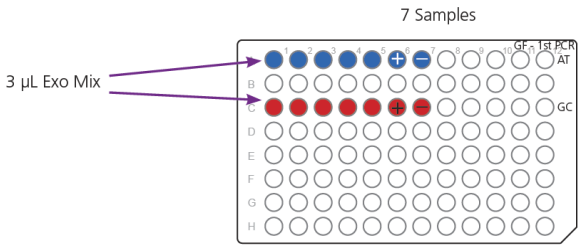
6. Resume the OncoScan Gap Fill protocol. Allow it to ramp down to the start of the 37°C step. Pause the protocol at the start of the 37°C step.

   Note: Ensure that the thermal cycler protocol is paused.

7. Centrifuge the **1st PCR Plate** for 30 seconds, then place it on a cooling block.

8. Remove the seal from the **1st PCR Plate**.

9. Transfer 3.0 µL of Exo Mix from the “EXO” strip tube to the **1st PCR Plate**. Pipet up and down to 3 times to rinse the tips.



10. Seal the **1st PCR Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.

11. Load the **1st PCR Plate** into the thermal cycler then resume the OncoScan Gap Fill protocol.

    Note: Ensure that the thermal cycler protocol has resumed and is running.

12. Start a timer for 20 minutes.

    Note: At 20 minutes, prepare the Cleavage Master Mix. At 30 minutes return to the thermal cycler to pause the protocol.

Stage 2G: Prepare and add the Cleavage Master Mix

1. Label a 1.5-mL tube “CM” and place it on ice.

2. Label a 7-well strip tube “CM” and place it on a cooling block.

3. Remove the Cleavage Enzyme (2 U/µL) from the freezer and place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.

4. Prepare the Cleavage Master Mix in the “CM” 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 5 Cleavage Master Mix.

Reagent and cap color	1 reaction	14 reactions (~25% overage)
Cleavage Buffer	25.0 µL	438.0 µL
Cleavage Enzyme (2 U/µL)	0.2 µL	3.5 µL
Total volume	**25.2 µL**	**441.5 µL**
Strip tube wells		**7**
Volume per strip tube well		60.0 µL

5. Vortex and centrifuge the Cleavage Master Mix, then place it on ice.

6. Aliquot 60.0 µL of the Cleavage Master Mix into wells 1—7 of the “CM” strip tube, then place it on a cooling block.

7. When the protocol reaches the end of the 95°C incubation, and ramps down to the start of the 37°C step, pause the protocol.

   Note: Ensure that the thermal cycler protocol is paused.

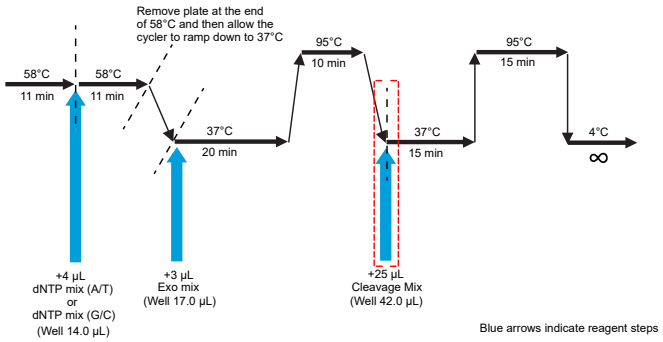
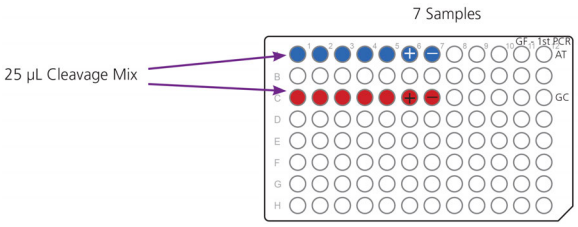


Figure 3 Cleavage Master Mix addition.

8. Remove the **1st PCR Plate** from the thermal cycler and place it on a cooling block for 1 minute. Centrifuge for 30 seconds, then place it on a cooling block.

9. Remove the seal from the **1st PCR Plate**.

10. Transfer 25.0 µL of Cleavage Master Mix from the “CM” strip tube to the **1st PCR Plate**. Pipet up and down to 3 times to rinse the tips.



11. Seal the **1st PCR Plate**, vortex, centrifuge for 1 minute, then place it on a cooling block.

12. Load the **1st PCR Plate** into the thermal cycler then resume the OncoScan Gap Fill protocol.

    Note: Ensure that the thermal cycler protocol has resumed and is running.

13. Start a timer for 25 minutes.

    Note: At 25 minutes, prepare the PCR Master Mix.

Stage 2H: Prepare and add the 1st PCR Master Mix

1. Label a 1.5-mL tube “PCR 1” and place it on ice.

2. Label a 7-well strip tube “PCR 1” and place it on a cooling block.

3. Remove the Taq Polymerase (5 U/µL) from the freezer and immediately place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.

4. Prepare the 1st PCR Master Mix in the “PCR 1” 1.5-mL tube labeled. Between additions, rinse tips, vortex, and centrifuge.

Table 6 1st PCR Master Mix.

Reagent and cap color	1 reaction	14 reactions (~25% overage)
◯ PCR Mix	24.4 µL	427.0 µL
◯ Taq Polymerase (5 U/µL)	0.56 µL	9.8 µL
Total volume	**24.9 µL**	**436.8 µL**
Strip tube wells		**7**
Volume per strip tube well		60.0 µL

5. Vortex and centrifuge the 1st PCR Master Mix, then place it on ice.

6. Aliquot 60.0 µL of the 1st PCR Master Mix into wells 1—7 of the “PCR 1” strip tube, then place it on a cooling block.

7. When the OncoScan Gap Fill protocol is complete and is at 4°C, remove the **1st PCR Plate** from the thermal cycler and place it on a cooling block for 1 minute. Centrifuge for 30 seconds, then place it on a cooling block.

8. Stop the OncoScan Gap Fill protocol. Start the OncoScan 1st PCR thermal cycler protocol. When the temperature reaches 60°C, pause the protocol.

   Note: Ensure that the thermal cycler protocol is paused.

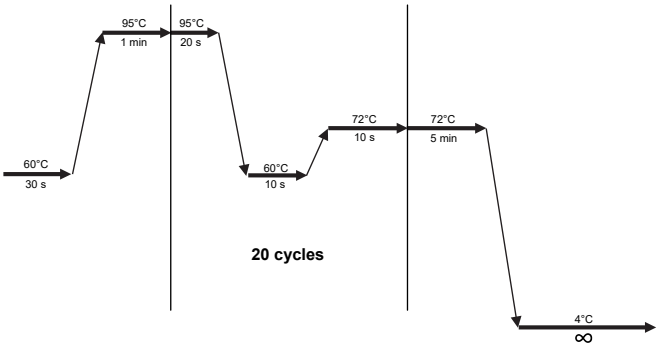
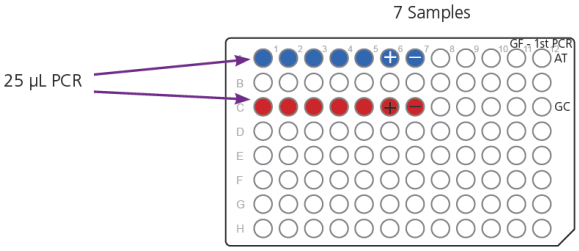


Figure 4 OncoScan 1st PCR thermal cycler protocol.

9. Remove the seal from the **1st PCR Plate**.

10. Transfer 25.0 µL of 1st PCR Master Mix from the “PCR 1” strip tube to the **1st PCR Plate**. Pipet up and down to 3 times to rinse the tips.



11. Seal the **1st PCR Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.

12. Load the **1st PCR Plate** into the thermal cycler then resume the OncoScan 1st PCR protocol.

    Note: Ensure that the thermal cycler protocol has resumed and is running

13. While the **1st PCR Plate** is in the thermal cycler, clean up the Pre-PCR Room benchtop.

Stage 2I: Complete the 1st PCR

1. When the OncoScan 1st PCR protocol is complete and is at 4°C, remove the **1st PCR Plate** from the thermal cycler and place it on a small tray of ice.

2. Stop the OncoScan 1st PCR protocol, then power off the thermal cycler.

3. Transfer the **1st PCR Plate** to the Post-PCR Room on ice.

IMPORTANT! To prevent contamination from PCR products, the **1st PCR Plate** Plate must remain sealed until it has been transferred to the Post-PCR Room. ***Do not*** open the seal, centrifuge, or store the **1st PCR Plate** in the Pre-PCR Room.

Stage 3: 2nd PCR and First QC Gel

Perform this Stage in the Post-PCR Room.

1. Fill the ice bucket with ice. Place 3 cooling blocks on ice.
2. Ensure that the **1st PCR Plate** is sealed. Vortex, centrifuge for 1 minute, then place it on a cooling block.
3. Thaw the PCR Mix.
4. Once thawed, vortex, centrifuge, and place it on ice.

Stage 3A: Prepare and add the 2nd PCR Master Mix

1. Power on the thermal cycler.
2. Start the OncoScan 2nd PCR thermal cycler protocol. When the temperature reaches 60°C, pause the protocol.

   Note: Ensure that the thermal cycler protocol is paused.

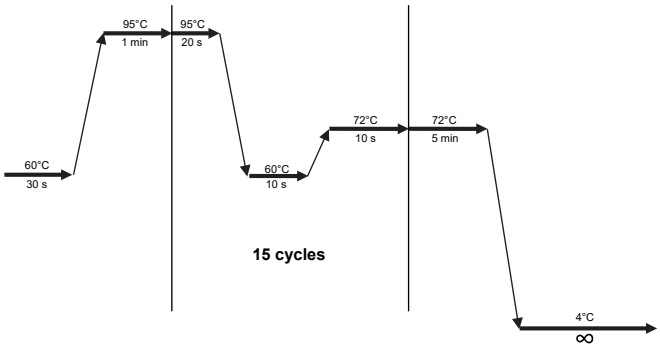


Figure 5 OncoScan 2nd PCR thermal cycler protocol.

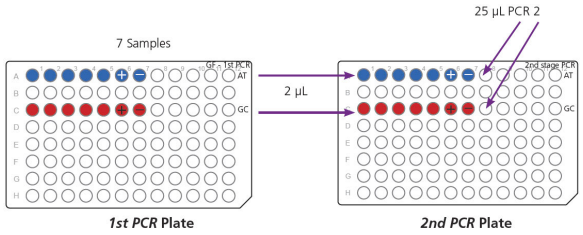
3. Label a 96-well plate “2nd PCR” and place it on a cooling block. This plate is called the **2nd PCR Plate**.
4. Label wells A1—A7 with a blue marker and wells C1—C7 with a red marker.
5. Label a 1.5-mL tube “PCR 2” and place it on ice.
6. Label a 7-well strip tube “PCR 2” and place it on a cooling block.
7. Remove the Taq Polymerase (5 U/µL) from the freezer and place it in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place it in the cooler.
8. Prepare the 2nd PCR Master Mix in the “PCR 2” 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 7 2nd PCR Master Mix.

Reagent and cap color	1 reaction	14 reactions (~25% overage)
◯ PCR Mix	24.4 µL	427.0 µL
◯ Taq Polymerase (5 U/µL)	0.56 µL	9.8 µL
Total volume	**24.9 µL**	**436.8 µL**

Reagent and cap color	1 reaction	14 reactions (~25% overage)
Strip tube wells		**7**
Volume per strip tube well		60 µL

9. Vortex and centrifuge the 2nd PCR Master Mix, then place it on ice.
10. Aliquot 60.0 µL of the 2nd PCR Master Mix into wells 1—7 of the “PCR 2” strip tube, then place it on a cooling block.
11. Transfer 25.0 µL of 2nd PCR Master Mix from the “PCR 2” strip tube to the **2nd PCR Plate**.
12. Remove the seal from the **1st PCR Plate**.
13. Transfer 2.0 uL of 1st PCR product from the **1st PCR Plate** to the **2nd PCR Plate**. Pipet up and down to 3 times to rinse the tips.



14. Cover the **1st PCR Plate** with an unopened seal until the First QC Gel.
15. Seal the **2nd PCR Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.
16. Load the **2nd PCR Plate** into the thermal cycler then resume the OncoScan 2nd PCR protocol.

    Note: Ensure that the thermal cycler protocol has resumed and is running.
17. While the OncoScan 2nd PCR protocol is running, perform the First QC Gel.

Stage 3B: Run the First QC Gel

1. Assemble the E-Gel™ Power Snap Plus Electrophoresis System.
2. Place an E-Gel™ Agarose Gel with SYBR™ Safe DNA Gel Stain, 4% on the benchtop.
3. Place Nuclease-Free Water (from different source), TrackIt™ Cyan/Orange Loading Buffer, and 25 bp DNA Ladder on the benchtop.
4. Prepare dilutions of the TrackIt™ Cyan/Orange Loading Buffer and the 25 bp DNA Ladder.
   a. Label a 15-mL tube “1:100 diluted Loading Buffer”. Add 100.0 µL of TrackIt™ Cyan/Orange Loading Buffer to 9.9 mL Nuclease-Free Water (from different source). Mix well, then store at room temperature.

b. Label a 1.5-mL tube “1:6 25 bp DNA Ladder”. Add 27.0 µL of 25 bp DNA Ladder to 135.0 µL of Nuclease-Free Water (from different source). Vortex, centrifuge, then place it on the benchtop.

5. Label a 96-well plate “GEL 1” and place it on a cooling block. This plate is called the **Gel 1 Plate**.

6. Label wells A1—A7 with a blue marker and wells C1—C7 with a red marker.

7. Add 16.0 µL of 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer to each labeled well of the **Gel 1 Plate**.

8. Remove the unopened seal from the **1st PCR Plate**. Transfer 4.0 µL of the 1st PCR product from the **1st PCR Plate** to the corresponding well of the **Gel 1 Plate**. Pipet up and down 3 times to rinse the tips.

9. Seal the **Gel 1 Plate**. Vortex, centrifuge for 1 minute, then place it on a plate storage rack.

10. Remove the comb from the gel and insert the gel into the electrophoresis device.

11. Remove the seal from the **Gel 1 Plate**.

12. Load 20.0 µL of each sample from the **Gel 1 Plate** into the gel wells.

13. Load 15.0 µL of the 1:6 diluted 25 bp DNA Ladder into the marker wells. Load 15.0 µL of the 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer into any remaining empty wells.

14. Select the number of wells and the type of E-Gel™ Agarose Gel being run. Set the duration to 15 minutes and start the run.

    Note: After 15 minutes, return to the E-Base™ device to inspect and capture an image of the gel. The 1st PCR sample wells should have a single band of ~120 bp. The negative control should not have a band. The image should look like following example. (Figure 6.)

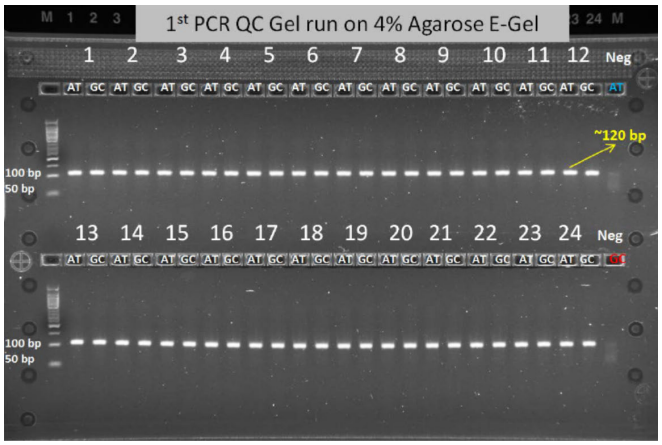


Figure 6 Example of a successful First QC Gel.

Stage 3C: Complete the OncoScan 2nd PCR protocol

1. When the OncoScan 2nd PCR protocol is complete and is at 4°C, remove the **2nd PCR Plate** from the thermal cycler.

2. Place it on a cooling block for 1 minute. Vortex, centrifuge for 1 minute, then place it on a cooling block.

3. Stop the OncoScan 2nd PCR protocol.

Stage 4: HaeIII Digest and Second QC Gel

Perform this stage in the Post-PCR Room.

1. Fill the ice bucket with ice. Place 3 cooling blocks on ice.

2. Thaw the Buffer B.

3. Once thawed, vortex, centrifuge, and place it on ice.

Stage 4A: Prepare and add the 2nd PCR Master Mix

1. Label a 96-well plate “HAE” and place it on a cooling block. This plate is called the **HAE Plate**.

2. Label wells A1—A7 with a blue marker and wells C1—C7 with a red marker.

3. Label a 1.5-mL tube “HAE” and place it on ice.

4. Label a 7-well strip tube “HAE” and place it on a cooling block.

5. Remove the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) from the freezer and place them in a cooler. Centrifuge for 1 second, then vortex 1 second. Centrifuge for 3 seconds, then place them in the cooler.

6. Prepare the HaeIII Master Mix in the “HAE” 1.5-mL tube. Between additions, rinse tips, vortex, and centrifuge.

Table 8 HaeIII Master Mix.

Reagent and cap color	1 reaction	14 reactions (~20% overage)
Buffer B	19.10 µL	321.0 µL
HaeIII Enzyme (10 U/µL)	0.40 µL	6.7 µL
Exo I Enzyme (20 U/µL)	0.50 µL	8.4 µL
Total volume	**20.0 µL**	**336.1 µL**
Strip tube wells		**7**
Volume per strip tube well		45.0 µL

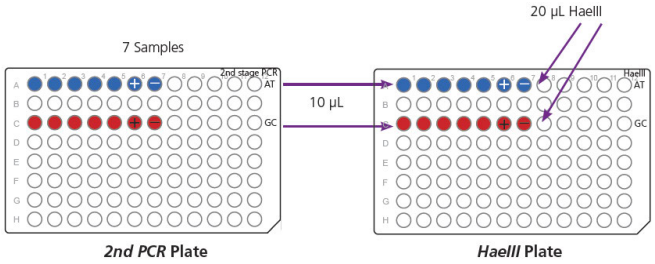
7. Vortex and centrifuge the HaeIII Master Mix, then place it on ice.

8. Aliquot 45.0 µL of the HaeIII Master Mix into wells 1—7 of the “HAE” strip tube, then place it on a cooling block.

9. Transfer 20.0 µL of HaeIII Master Mix from the “HAE” strip tube to the **HAE Plate**.

10. Remove the seal from the **2nd PCR Plate**.

11. Transfer 10.0 µL of 2nd PCR product from the **2nd PCR Plate** to the **HAE Plate**. Pipet up and down 3 times to rinse the tips.



12. Seal the **HAE Plate**. Vortex, centrifuge for 1 minute, then place it on a cooling block.

13. Load the **HAE Plate** into the thermal cycler and start the OncoScan HaeIII protocol.

    Note: Ensure that the thermal cycler protocol is running.

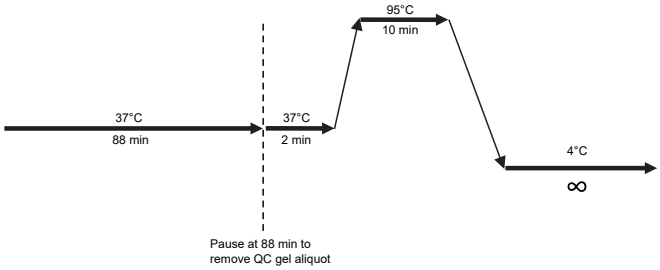


Figure 7 OncoScan HaeIII thermal cycler protocol.

14. Start a timer for 85 minutes.

IMPORTANT! After 85 minutes, return to the thermal cycler to pause the protocol and remove an aliquot for the Second QC Gel. The OncoScan HaeIII protocol must be paused, and the aliquot taken before the thermal cycler block temperature ramps to 95°C.

Stage 4B: Remove an aliquot of the HaeIII Product

1. Label a 96-well plate "GEL 2" and place it on a cooling block. This plate is called the **Gel 2 Plate**.

2. Label wells A1—A7 with a blue marker and wells C1—C7 with a red marker.

3. Place the previously prepared 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer and the 1:6 diluted 25 bp DNA Ladder on the benchtop.

4. Add 16.0 µL of 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer to each labeled well of the **Gel 2 Plate**.

5. When the protocol reaches 88 minutes, and the temperature is at 37°C, pause the protocol.

   Note: Ensure that the thermal cycler protocol is paused.

6. Remove the **HAE Plate** from the thermal cycler and place it on a cooling block for 1 minute. Centrifuge for 30 seconds, then place it on a cooling block.

7. Remove the seal from the **HAE Plate**.

8. Transfer 4.0 µL of the HaeIII product from the **HAE Plate** to the corresponding well of the **Gel 2 Plate**. Pipet up and down 3 times to rinse the tips.

9. Seal the **HAE Plate**, then load the **HAE Plate** into the thermal cycler. Resume the OncoScan HAEIII protocol.

   Note: Ensure that the thermal cycler protocol has resumed and is running

10. Seal the **Gel 2 Plate**. Vortex, centrifuge for 1 minute, then place it on a plate storage rack.

Stage 4C: Run the Second QC Gel

1. Assemble the E-Gel™ Power Snap Plus Electrophoresis System.

2. Place an E-Gel™ Agarose Gel with SYBR™ Safe DNA Gel Stain, 4% on the benchtop.

3. Remove the comb from the gel and insert the gel into the electrophoresis device.

4. Remove the seal from the **Gel 2 Plate**.

5. Load 20.0 µL of each sample from the **Gel 2 Plate** into the gel wells.

6. Load 15.0 µL of the 1:6 diluted 25 bp DNA Ladder into the marker wells. Load 15.0 µL of the 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer into any remaining empty wells.

7. Select the number of wells and the type of E-Gel™ Agarose Gel being run. Set the duration to 15 minutes and start the run.

   Note: After 15 minutes, return to the E-Base™ device to inspect and capture an image of the gel. The HAEIII product sample wells should have a predominant pattern of double bands at 40 bp and 70 bp. These double bands indicate that the 2nd PCR reaction and the HaeIII digestion were successful. (Figure 8.)

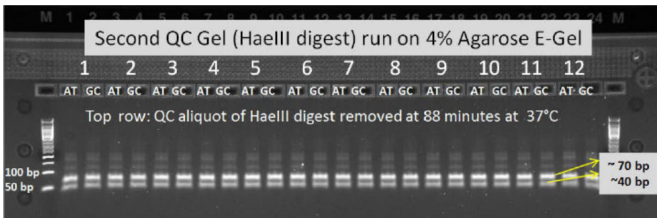


Figure 8 Example of a successful Second QC Gel.

Stage 4D: Complete the HaeIII protocol

1. When the OncoScan HaeIII protocol is complete and is at 4°C, remove the **HAE Plate** from the thermal cycler.

2. Place it on a cooling block for 1 minute. Vortex, centrifuge for 1 minute, then place it on a cooling block.

3. Stop the OncoScan HAEIII protocol.

Stage 5: Hybridization

Perform this stage in the Post-PCR Room.

1. Power on the GeneChip™ Hybridization Oven 645 and set it to 49°C and 60 rpm.
2. Allow the oven to operate for 30 minutes before arrays are loaded.
3. Fill the ice bucket with ice. Place 3 cooling blocks on ice.
4. Thaw the Nuclease-Free Water and the Hybridization Mix.
5. Once thawed, vortex, centrifuge, and place them on ice.

Stage 5A: Register and label the arrays

1. Place 2 arrays for each sample on the benchtop to warm to room temperature.

   Note: The Negative Control will not be hybridized.

2. Register each array using the Applied Biosystems™ GeneChip™ Data Collection Software (GCDC). During registration, the sample and the channel (AT or GC) are associated with the array barcode.

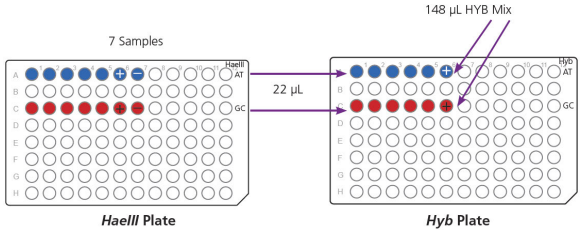
Stage 5B: Prepare the Hybridization Plate

1. Label a 96-well plate "HYB" and place it on a cooling block. This plate is called the **Hyb Plate**.
2. Label wells A1—A6 with a blue marker and wells C1—C6 with a red marker.
3. Label a 15-mL tube "HYB" and place it on ice.
4. Prepare the Hybridization Master Mix in the "HYB" 15-mL tube.

Table 9 Hybridization Master Mix.

Number of samples (includes Positive Control)		6
Reagent and cap color	**1 array**	**12 arrays**
◯ Nuclease-Free Water	30.0 µL	0.504 mL
◯ Hybridization Mix	118.0 µL	1.982 mL
Total volume	**148.0 µL**	**2.486 mL**

5. Vortex the Hybridization Master Mix continuously for 3 seconds. *Repeat* the vortex step 2 more times.
6. Transfer 148.0 µL of the Hybridization Master Mix from the "HYB" 15-mL tube to the **Hyb Plate**.
7. Remove the seal from the **HAE Plate**.
8. Transfer 22.0 µL of the HaeIII product from the **HAE Plate** to the **Hyb Plate**. Pipet up and down 3 times to rinse the tips.



9. Seal the **Hyb Plate**, then vortex.
10. *Repeat* the **Hyb Plate** vortex, centrifuge for 1 minute, then place it on a cooling block.

IMPORTANT! Ensure that the plate is vortexed to mix the sample and hybridization buffer well.

Stage 5C: Hybridize the arrays

1. Load the **Hyb Plate** into the thermal cycler and start the OncoScan Hybridization protocol.

   Note: Ensure that the thermal cycler protocol is running.

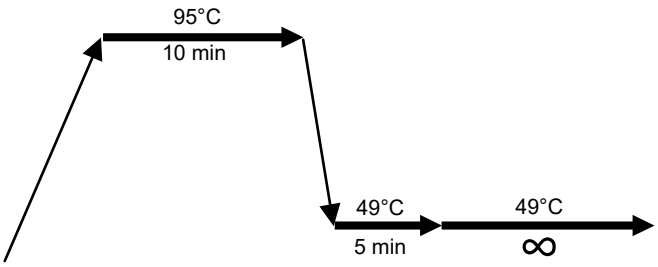


Figure 9 OncoScan Hybridization thermal cycler protocol.

2. Apply two 1/2" Tough-Spots™ label dots on the top edge of each array. Vent each array with a 200 µL pipette tip.
3. When the OncoScan Hyb Protocol reaches the 49°C ∞ step, open the lid. Keep the **Hyb Plate** in the thermal cycler.
4. Use a blade to cut the seal of the **Hyb Plate** between rows.
5. Aspirate 160.0 µL of a Hybridization Cocktail from the **Hyb Plate**, then immediately inject it into the corresponding array.
6. Cover the septa of each array with the 1/2" Tough-Spots™ label dots. Immediately load the array into the GeneChip™ Hybridization Oven 645.

IMPORTANT! Only process 4 arrays at a time. Do not allow injected arrays to sit at room temperature for more than 1 minute.

7. *Repeat* step 4 through step 6 until all arrays are loaded into the hybridization oven.

8. Allows the arrays to hybridize for 16—18 hours at 49°C and 60 rpm.

   IMPORTANT! The hybridization time, temperature, and rotational speed are optimized for this product and must be stringently followed.

9. When all arrays are in the hybridization oven, clean up the Post-PCR Room benchtop.

Stages 6 and 7: Wash, stain, and scan the arrays

Perform this stage in the Post-PCR Room.

1. Prepare the stains:
   a. Mix Stain 1, Stain 2, and Array Holding Buffer by gently inverting them 10 times.
   b. For each array, aliquot:
      - 500 µL of Stain 1 into an amber tube.
      - 500 µL of Stain 2 into a clear tube.
      - 1,000 µL of Array Holding Buffer into a blue tube.
2. Prepare the GeneChip™ Fluidics Station 450:
   a. Place full bottles of Wash A, Wash B , and DI water on the fluidics station.
   b. Empty the Waste Bottle and place it on the fluidics station.
3. Prime the fluidics station. When the protocol is complete, eject the wash block to avoid a sensor timeout.
4. Load the Stain Buffers and the Array Holding Buffer onto the fluidics station.
5. After 16—18 hours, remove arrays from the hybridization oven. Remove the Tough-Spots™ label dots, then load the arrays into the fluidics station.
6. Run the **OncoScan.Universal** fluidics protocol.
7. When the protocol is complete, remove the arrays from the fluidics station. Check for bubbles.
8. Cover the septa of each array with 3/8" Tough-Spots™ label dots. Begin scanning the arrays.
9. When the hybridization of all arrays is complete, power off the oven.
10. When the washing and staining of all arrays is complete, perform the **Shutdown** protocol, then power off the fluidics station.
11. When all arrays have been scanned, close the GCDC software, then power off the scanner.

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Products manufactured at this site:

OncoScan™ CNV Plus Reagent Kit

Affymetrix Pte Ltd | 7 Gul Circle #2M-01 | Keppel Logistics Building | Singapore 629563

Products manufactured at this site:

OncoScan™ CNV Plus Array

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029389

Revision	Date	Description
A.0	5 July 2023	Initial release.

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